Common Themes in Microbial Pathogenicity

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INTRODUCTION

Microbial pathogenicity has been defined as "the biochemical mechanisms whereby microorganisms cause disease" (233). Not all pathogens have an equal probability of causing infection and disease. (In this review, the term *infection* will be used to describe successful persistence or multiplication of a pathogen on or within the host, while *disease* will be used to describe an infection which causes significant overt damage to the host.) While some pathogens regularly cause disease in a proportion of non-immune individuals with intact host defense systems, others do not. For example, *Pseudomonas aeruginosa* can infect compromised patients and cause overwhelming disease but spares those with intact host defenses. Probably any microorganism which has the capacity to sustain itself in humans will occasionally cause disease in compromised individuals and act as an opportunistic pathogen. Thus, infection and disease are as dependent on the host as on the microorganism.

The usual outcome of a microbial infection is sufficient

multiplication by the pathogen to secure its establishment within the host by transient or long-term colonization or to bring about its successful transmission to a new susceptible host. Disease is an inadvertent and unfavorable outcome of such a microbial infection. It is important to recognize that a microorganism can be exceptionally equipped to cause infection and not cause disease. Circumstances occasionally dictate that disease regularly results from bacterial infection, but this is not usually the case.

We are just beginning to understand the molecular basis of microbial pathogenicity. At present, there are only a few examples for which the complete biochemical mechanisms are thought to be known. These examples are limited to toxins, such as diphtheria and tetanus, which act as single determinants to produce disease. Even in these cases, however, the actual contribution of the toxin to the pathogenesis of infection remains poorly defined. Microbial pathogenesis is usually complex and multifactorial. Pathogens have several biochemical mechanisms which may act individually or in concert to produce infection and disease. Removal of any one of these components may or may not render the organism avirulent. Furthermore, microbiologists have often neglected the complex role of the host. Only recently have we begun to pay more attention to animal models and to exploit

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the knowledge of cell biology and immunology in our studies of microbial pathogenesis.

The detailed examination of a variety of bacterial virulence factors is now possible. Recombinant deoxyribonucleic acid (DNA) techniques permit the precise genetic manipulation of single or multiple virulence genes. These genetic sequences can be transferred to well-defined background strains to assess their contribution to pathogenicity. DNA probes permit determination of the extent to which various virulence factors are present throughout the microbial world. The evolution of cell and organ culture techniques has provided us with new methods to characterize host-parasite interactions and to study intracellular pathogens better. It is not within the scope of this review to summarize our current knowledge of all mechanisms of microbial pathogenesis. Nor can we hope to be comprehensive when writing about such a broad discipline as microbial pathogenicity. This information has become far too vast, and general overviews are available from other sources (32, 167, 233–236). It has become apparent from studies with pathogenic organisms that several common themes are repeatedly used by pathogenic microbes for infection; these themes are the focus of this review. This is not to say that virulence factors are necessarily conserved; instead, different microorganisms have evolved separate and distinct mechanisms for overcoming common host or environmental barriers to infection. For example, although there are a limited number of ports of entry into a host, many diverse pathogenic bacteria are capable of entering at each site. Interaction with host epithelial surfaces is a process shared by many pathogenic organisms, yet distinct alternative mechanisms exist for these interactions. The host immune system is a formidable deterrent that must be breached or avoided by most pathogenic organisms. It is worthwhile to compare and contrast the various tactics used by different organisms to solve this common obstacle. It is also useful to understand the pathogenesis of infection as new therapeutic and preventative schemes are being weighed. In this review, we have attempted to compile examples of some of the mechanisms utilized by pathogenic bacteria to overcome host barriers which illustrate various themes in microbial pathogenicity. Our choice of topics and the detail with which we discuss any subject reflect our own interests and not necessarily the relative importance of the subject to understanding microbial virulence. Nevertheless, we hope that the information presented here affords useful insights into the evolution and molecular diversity associated with host-parasite interactions. Finally, as we discover the ways by which microorganisms outwit their hosts, we also learn more about related host processes.

ENTRY AND ADHERENCE

Animal hosts have various protective mechanisms to prevent microbial entry. At the same time, animals must also maintain contact with the environment to exchange air, ingest food, and eliminate body wastes. Pathogenic organisms have evolved mechanisms to capitalize on these sites of environmental contact as points of entry (167). The skin is the predominant host barrier which excludes most microorganisms. This organ system can be damaged by trauma of various types, allowing organisms with pathogenic potential, such as the staphylococci, to enter. Organisms which enter by this mechanism are usually present on the skin prior to injury. One natural mechanism to bypass the skin barrier is direct inoculation into the body by arthropod bites. Several

pathogenic bacteria use this route of entry, including *Yersinia pestis* (plague) and *Rickettsia* spp. (typhus and spotted fevers). These organisms spend part of their life cycles within the arthropod, an environment vastly different from that found within a human or other animal host. Another means in the modern world by which bacteria and other infectious agents bypass the skin is inoculation by needles or direct implantation of contaminated foreign bodies within the host. For example, *Staphylococcus epidermidis*, an organism usually found on skin, can infect substantial numbers of individuals who have received a prosthetic implant.

Other sites of entry into human hosts include the digestive tract, the respiratory tract, the urogenital tract, and the conjunctiva. Specialized cells in each of these anatomic sites provide mechanical cleansing activities (such as peristalsis and blinking) to remove unwanted particles, including microorganisms. The surfaces of the cells in contact with the environment are also bathed by antimicrobial substances. Organisms which infect these regions have developed specific tissue adherence mechanisms which overcome the constant presence of cellular disinfection activities (discussed below). Each host surface is the target for a set of pathogenic bacteria which often use these areas as sites of multiplication as well as for entry into other host locales. These organisms are highly adapted for their unique niche, and this is usually reflected by the molecular structure and function of their specialized adherence factors. In addition, the microbial cell envelope is adapted for survival at the target niche and provides protection against local host defense systems.

We still know relatively little about the microbial factors essential to ensure infectious transmission from host to host. Presumably, bacteria have evolved mechanisms to take advantage of the existing avenues of contacts between hosts. Dissemination by aerosols is a common mechanism of transmission of respiratory pathogens such as *Bordetella pertussis* and *Mycobacterium tuberculosis*. However, successful transmission by this means requires that a number of criteria be met, including resistance to atmospheric exposure and drying.

The burden upon the pathogen which follows a fecal-oral route is substantial. The organism must be able to tolerate life outside the mammalian host for variable periods of time. Upon ingestion, the organism is summarily exposed to higher temperatures, extremes of pH, different available nutrients, high concentrations of bile salts, digestive enzymes, etc. Some enteric pathogens have learned to overcome the strong waves of peristalsis within the small bowel and to establish themselves in this niche, despite the presence of competitive bacterial populations in the colon. Then, after intraluminal or intracellular multiplication, enteric pathogens are once more expelled into the external environment to begin the cycle again.

Sexually transmitted pathogenic organisms are ordinarily transmitted by direct inoculation onto new mucosal surfaces. This microbial strategy for survival avoids life in an external environment, but it is not without its own special set of requirements to overcome changing pH, mucus obstruction, anatomic barriers, local antibody, and phagocytic cells.

Thus, ample opportunities exist for microorganisms to move from the environment to hosts or between hosts. A major prerequisite for the organism is to survive the environment between infections. Although the necessity for pathogens to grow in or at least tolerate several different environments has been largely ignored by medical microbiologists, we are beginning to identify bacterial regulatory

mechanisms which are uniquely adapted to a pathogenic "lifestyle." Specialized pathogenic traits and regulatory mechanisms may be induced only when the organism encounters a host. It has been recognized for some time that bacteria grown in broth in the laboratory may be inappropriate for the study of pathogenic factors (232). Only recently has this situation been taken into account experimentally.

The first major interaction between a pathogenic microorganism and its host entails attachment to a eucaryotic cell surface. Some microorganisms multiply at and remain on the surface of the host. Other organisms use attachment as the first essential step before proceeding to deeper tissue or other locations. If one wishes to inhibit a pathogenic organism from colonizing and establishing an infection, blocking initial attachment is a logical place to start.

The attachment stage is the best characterized of host-parasite interactions because it has been one of the easiest to address experimentally (74, 125, 202). In several cases the biochemistry of the factors involved in bacterial adherence have been thoroughly analyzed. The precise contribution of these adhesins to microbial pathogenicity has been surprisingly difficult to define. Many microbes express several distinct and alternative means of cell attachment. These alternative mechanisms may be expressed under different environmental and host conditions or even at different host surfaces. Hence, several adherence mechanisms acting collaboratively may define where a specific pathogen will colonize and begin to cause an infection. One supposes that microbial attachment mechanisms are usually designed to interact with receptors characteristic of a given host surface. The potential number of distinctive receptor molecules that exist as targets for microbial adherence to host surfaces are presumably as diverse as the available host surfaces. Yet, only a few appear to be targeted by pathogenic bacteria.

In its simplest form, microbial adherence requires the participation of two factors: a receptor and an adhesin. The receptors defined thus far are usually specific carbohydrate residues on the eucaryotic cell surface. The bacterial adhesin is typically a protein structure on the bacterial cell surface which interacts with the host cell receptor.

Fimbriae (Pili) as Adhesins

Type 1 pili. Many species of the *Enterobacteriaceae* family possess a prominent, morphologically similar fimbrial appendage which enables them to bind to D-mannose residues on eucaryotic cells (44, 53). These so-called common pili or type 1 fimbriae were initially thought to be composed of identical repeating subunits of about 17 to 21 kilodaltons (kDa). It is now recognized that minor proteins are also a part of the fimbrial structure. New evidence indicates that the D-mannose-binding site is not located in the major pilin structural unit but rather resides in a minor protein (about 28 to 31 kDa) located at the tips or inserted periodically along the length of the fimbriae (168, 175, 261). While the major structural fimbrial protein exhibits considerable variation among different enteric species, the minor tip adhesin is conserved among a broad representation of type 1 fimbriated members of the *Enterobacteriaceae* (2). The same motif is exhibited by other fimbrial systems operative in *Escherichia coli*. Conserved tip proteins which recognize eucaryotic carbohydrate receptors other than D-mannose are found for the Pap (pyelonephritis-associated) pili [which recognize the disaccharide α-Gal (1→4) β-Gal (107, 130, 176, 180)] and S fimbriae, which recognize sialic acid-containing glycoconjugates (169).

A single *E. coli* strain can express several distinct types of fimbriae or adhesins encoded by distinct regions on the chromosome and plasmids (140, 261). This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces. Several lines of evidence suggest that many of the adhesive fimbriae from *E. coli* have evolved from a common primordial ancestor (124, 170). There is amino acid homology at the amino and carboxyl termini of various pilin subunits, including the chromosomally encoded type 1 and Pap fimbriae as well as the plasmid-mediated CFA1, K88, and K99 pili; moreover, most pilin molecules are of similar subunit size (170). The genes involved in pilus biosynthesis are also encoded by a similar number of accessory proteins and are often arranged in a similar genetic order. It was recently demonstrated that uropathogenic *E. coli* can express two pili, Pap-G and Prs-G, which are serologically identical, yet possess different binding specificities (142). By genetically varying the minor tip protein adhesin, this organism gains the ability to bind to alternative receptors. It is not certain that the structural fimbrial subunit is always devoid of adhesive capabilities of its own. However, the general theme for enterobacterial fimbriae may be that either the major structural unit acts as a scaffolding for a distinct minor adhesin or an immunorecessive domain of the structural unit has receptor-binding specificity.

E. coli isolates from pyelonephritis can exhibit at least three distinct adhesins, type 1, X, and P fimbriae. A high percentage of strains isolated from pyelonephritis encode an operon which specifies both the P-pilus structure and the P adhesin recognizing the digalactoside; most strains also express type 1 pili. However, 10 to 15% of the uropathic *E. coli* recognize receptors other than the digalactoside- and mannose-binding specificities, and these have been referred to as X adhesins (264). Some of these putative X adhesins have now been shown to have S specificity (129) for sialyl-galactosides or have M-blood-group glycophorin A specificity (203, 264). Another X adhesin is now identified in *E. coli* pyelonephritis strains as an afimbrial adhesin (133, 134) which mediates adherence to human transitional and squamous epithelial cells. The operon expressing afimbrial adhesin is composed of five distinct genes of which one, *afaE*, encodes the 16,000-Da hemagglutinin adhesin which recognizes a receptor distinct from S, M, or Pap fimbriae. Other *E. coli* strains, either from urinary tract infection or those associated with diarrheal disease, possess sequences highly related to the *afaA-D* genes but unrelated to the adhesin *afaE* product. These strains did express a functional operon but contained a structural adhesin-coding gene unrelated at the DNA level to the *afaE* gene. Once again, we see a common theme of providing a basic structural scaffolding upon which immunologic variation can be built or upon which quite different adhesive specificities may be utilized. Subsequent studies (A. Labigne-Roussel, personal communication) have shown that *afa* operon distribution is not restricted just to uropathic *E. coli* but is found in a number of pathogenic bacteria, including enteropathogenic strains. It is likely that these organisms express these and possibly other alternative types of fimbriae to facilitate adherence to different surfaces or under different conditions.

The role of type 1 fimbriae in the pathogenesis of infection has been difficult to discern (74, 202). Many members of the *Enterobacteriaceae* prefer different niches within human hosts, yet they express functionally identical type 1 fimbriae. Nonfimbriated strains of *Salmonella typhimurium* are as virulent as fimbriated strains when fed orally to mice (52). It

was suspected that type 1 pili would recognize a eucaryotic receptor which contained mannose residues, and this has been confirmed (178, 205, 227). Bacteria expressing type 1 pili also bind to the Tamm-Horsfall protein, a mannose-containing glycoprotein produced in the kidney and released into urine (111, 132, 182). This secreted protein may protect the kidney from bacterial infection by inhibiting binding of type 1 pili to its receptor.

It has been suggested that type 1 fimbrial structures play a significant role in *E. coli* colonization of the urinary tract and in colonization of the large bowel. However, type 1-piliated *E. coli* K1 strains typical of those isolated from neonatal meningitis are at a marked disadvantage in mice; they are rapidly eliminated without causing a progressive infection. In contrast, the same strains expressing S fimbriae are highly virulent in this animal model (177). The capacity of these bacteria to become S-fimbriated was induced in vitro and in vivo by a dialyzable serum component. Along a similar vein, Bloch and Orndorff (personal communication) have shown that an *E. coli* K1 strain deleted for its capacity to express type 1 pili could colonize the bowel and cause disease; however, this mutant does not colonize the oropharynx, although the type 1-fimbriated parental strain does so readily. A recent report suggests that *E. coli* colonize the oropharynx of normal human neonates more commonly than had been supposed previously (9). Despite the conflicting data for type 1 fimbriae, these appendages are potentially useful or even required by some organisms for the colonization of the vaginal and bladder mucosal surfaces (202). There is evidence, acquired from epidemiologic and animal studies, that P fimbriae (and X adhesins) are important for adhering to tissues of the urogenital tract and are required for ascending infections of the kidneys, in addition to their role in the colonization of the vagina and perhaps the urethra (202). The interaction and specific roles of these various adhesins in urinary tract infections are complex and have not been clearly resolved.

It seems certain that most pathogenic bacteria possess a repertoire of adhesins that may be called upon during their life cycle. While uropathic *E. coli* commonly possess two or more different fimbriae, they are not usually expressed simultaneously. In the laboratory, it is well known that type 1 fimbriae are expressed in liquid media while P pili are best expressed following growth on solid media. For other pili types, it is understood that certain conditions are more favorable than others for expression. Hence, as we point out later, the bacterial adhesins follow a common theme in that their expression is a reflection of a broadly orchestrated series of events that occur during the pathogenesis of infection. Adherence is important not only during the initial encounter between the pathogen and its host, but also throughout the infection cycle.

N-Methylphenylalanine pili. Another type of pili found in diverse gram-negative organisms are the *N*-methylphenylalanine pili. These pili are characterized by a pilin subunit which contains a methylated phenylalanine at its amino terminus (77) followed by a highly conserved region of 25 to 30 hydrophobic amino acids. The signal sequence of the genes encoding these pili is six to seven residues in length. These pili are found in *Pseudomonas* (183), *Neisseria* (159), *Moraxella* (147), *Bacteroides* (54–56), and *Vibrio* (253) species. In at least one case, it has been established that these pili are virulence determinants (275), and the receptor(s) for another of these pili (*Neisseria gonorrhoeae*) is thought to be an oligosaccharide (247). The conserved amino terminus of these molecules has enabled researchers to express *Bac-*

teroides nodosus pili in *P. aeruginosa* (57, 58, 148), but multispecies pilus vaccines seem unlikely to be developed. The conservation of the amino terminus of these structural proteins probably reflects a conserved mechanism for pilus biosynthesis. The shared region of homology between diverse species is involved in pilin subunit-subunit interactions. The remaining portion of the pilin protein is not conserved between species, and it seems likely that these divergent regions contain the binding specificity. It may be more than a coincidence that the *N*-methylphenylalanine pili are a common theme in microorganisms which are localized at the mucosal surface.

Other Adhesins

Several other nonfimbrial adhesins have been reported. Filamentous hemagglutinin from *Bordetella pertussis* (219, 268) and mannose-resistant hemagglutinin from *Salmonella typhimurium* (98, 118) are two such examples. They all mediate adherence to host surfaces, yet their molecular structures and their cellular targets are different. Later in this review, we discuss another class of nonfimbrial adhesins, the invasion proteins, which not only mediate bacterial attachment to the host surface but also provide the key for entry of the microorganism into the host cell.

Fibronectin and Staphylococcal and Streptococcal Adherence: One Receptor Molecule but Two Adhesins

Fibronectin is a large, multifunctional, extracellular matrix and plasma glycoprotein which promotes numerous adherence functions in mammalian cells (155, 199). This molecule also adheres in large quantities to mucosal surfaces. Two of the best known pathogenic bacteria, *Streptococcus pyogenes* (group A streptococci) and *Staphylococcus aureus*, adhere to fibronectin on epithelial cell surfaces (16, 200). Although these two organisms adhere to the same molecule under similar conditions, they use different mechanisms. The group A streptococcal adhesin, lipoteichoic acid, is anchored to proteins on the bacterial surface, including the M protein. Lipoteichoic acid mediates the attachment of these bacteria to the amino terminus of fibronectin through the glycolipid end of lipoteichoic acid (16, 17, 50). *Staphylococcus aureus* also binds to the amino terminus of fibronectin, but at a distinct site (16, 200). A large fibronectin-binding protein has been identified in *Staphylococcus aureus* (59, 76) and cloned into *E. coli* (71). Although the characterization of this molecule is in progress, it is obvious that it is different from lipoteichoic acid, suggesting that *Staphylococcus aureus* and group A streptococci use different mechanisms to adhere to the same receptor on epithelial surfaces.

Treponema pallidum (the causative agent of syphilis) is another pathogenic organism which binds fibronectin (255), but at a different site. Three related surface adhesins of *T. pallidum* (P1, P2, and P3) bind to a four-amino-acid sequence (RGDS) of the cell-binding domain of fibronectin (189, 256, 257). (As discussed later, integrins on the eucaryotic cell surface also bind to this region of fibronectin.) The role of fibronectin binding in the pathogenesis of syphilis has not been resolved; this organism may use fibronectin to attach to host surfaces, or it may coat itself in fibronectin to avoid the host immune system (14, 255).

Role of Bacterial Chemotaxis in Pathogenesis

Many motile bacteria have the capacity to move towards nutrients (chemotaxis), thus entering a more favorable envi-

TABLE 1. Comparison of the invasion strategies used by *Salmonella*, *Shigella*, and *Yersinia* species

Species	Cell type entered in gut	Host micro-filaments required for entry	Endosome acidification needed for entry or intracellular replication	Intracellular location	Vacuoles with bacteria coalesce	Intracellular replication in epithelial cells	Bacterial metabolic activity required for entry	Adherence to epithelial cell surfaces at 4°C	Plasmid required for entry
Salmonella[a]	Epithelial and Peyer's patches (M cells)	Yes	No	Vacuole	Yes	Yes	Yes	No	No
Shigella	Mucosal epithelial	Yes	No	Cytoplasm		Yes	Yes	?	Yes
Yersinia[b]	Peyer's patches	Yes	No	Vacuole	No	Slow, varies with cell line	No	Yes	No

[a] Most *Salmonella* species except *S. typhi*.

[b] *Y. pseudotuberculosis* and *Y. enterocolitica*.

ronment. Although some pathogenic organisms are nonmotile (the highly virulent shigellae are one example), the capacity to move towards a host surface has obvious benefits. *Vibrio cholerae* motility greatly enhances their association with human intestinal mucosa (5, 73, 75), perhaps by propelling the organism towards the intestinal surface.

Chemotaxis may also contribute to *Salmonella* entry into eucaryotic cells. Uhlman and Jones (262) demonstrated that a diffusible attractant that was released from HeLa cells greatly enhanced the collision frequency between *Salmonella typhimurium* and the epithelial cells. If the bacteria were centrifuged onto the monolayer, the chemotactic dependence for adherence was not required. A recent study of the factors required by *Salmonella typhi* for invasion of HeLa cells suggested that flagella, motility, and chemotaxis were all necessary (139). However, in most bacteria the role of chemotaxis towards host surfaces has not been addressed and remains poorly characterized.

INVASION OF HOST CELLS

Entry into host cells is a specialized strategy for survival and multiplication utilized by a number of pathogens (171). Besides avoiding the host defense immune system, intracellular localization places the organism in an environment potentially rich in nutrients, yet devoid of competing organisms. However, intracellular life is not free of difficulty. Invasive pathogens face a different set of requirements than pathogenic organisms, which live their life free in the environment or bound to host surfaces. The biology of intracellular parasites has been extensively reviewed elsewhere (171), and our discussion serves only to supplement that excellent review.

To pursue an intracellular lifestyle, an organism must first penetrate the eucaryotic cell surface barrier and gain entry (invasion) into the host cell. It appears that most invasive pathogens exploit existing eucaryotic internalization pathways. For example, if an organism adheres tightly to a receptor on a eucaryotic cell and this receptor is then internalized, the bacteria may also gain entry into the host cell. Simple adherence is not sufficient; bacteria which tightly adhere to animal cells by means of type 1 or Pap pili are not internalized (162). Possibly because of the large size of invasive organisms (compared with normal endocytosed particles), there is usually cytoskeletal rearrangement accompanying bacterial invasion. Internalization of most pathogenic organisms is inhibited by cytochalasins, agents which inhibit microfilament function, but microtubules and intermediate filaments do not appear to be involved in

bacterial invasion (65). Once inside the host cell, the organism must be able to survive, multiply, and ultimately escape from the host cell; intracellular multiplication usually takes place to some degree but is not necessarily a requirement. At least four genera of the *Enterobacteriaceae* are invasive: *Salmonella*, *Shigella*, *Escherichia*, and *Yersinia*. Although they are related taxonomically, these organisms use three different, distinct invasive schemes (Table 1) which we discuss here for comparative purposes.

Shigella

Enteroinvasive *E. coli* and *Shigella* spp. use the same mechanisms to enter into eucaryotic cells and, for this discussion, are considered equivalent. These organisms enter humans by the fecal-oral route and proceed through the stomach (surviving the low pH) to the lower bowel, where they interact with the intestinal mucosa. *Shigella* species typically invade the mucosal epithelial cells of the colon (72, 95, 128, 252). The infection is usually confined to the superficial layers of the intestinal mucosa, and the organisms spread to other surface epithelial cells and cause much tissue damage (ulceration), fluid secretion, and inflammation, producing the clinical manifestations of dysentery (diarrhea with blood and mucus). It is less well appreciated that malnourished individuals may show evidence of invasive disease beyond the colonic mucosa, including bacteremia (248).

Shigella spp. enter cultured animal cells by a process of induced endocytosis which requires host energy expenditure and the active participation of host microfilaments but not microtubules (45, 46, 65, 66, 80, 96). Also, *Shigella* spp. must be metabolically active to enter into host cells, as bacteria treated with ultraviolet radiation or kanamycin do not invade (93). *Shigella* spp. are internalized within a host membrane-bound inclusion. The membrane enclosing the bacterium is lysed soon after bacterial entry (within 15 min), and the organism is released into the host cytoplasm. Escape from the endocytic vacuole is mediated by a virulence plasmid-encoded product, the contact hemolysin, which presumably lyses the host membrane which encloses the bacterium (215). (The term *hemolysin* may be an unfortunate choice, but was given to this product since *Shigella* spp. can lyse erythrocytes when placed in direct contact with them.) Release from the endocytic vacuole is an essential process for *Shigella* virulence, as intracellular replication does not occur when this activity is disrupted. A similar theme of bacterial release from an endocytic vacuole has been reported for the gram-positive organism *Listeria monocyto-*

genes (78, 131, 197). This organism produces listeriolysin, a secreted hemolytic factor, which is required for escape to the cytoplasm from the initial inclusion vacuole. *L. monocytogenes* mutants lacking this hemolysin cannot replicate intracellularly and are avirulent in a mouse model (197).

Once free in the cytoplasm, *Shigella* spp. inhibit host protein synthesis and multiply rapidly (47, 80, 214). Approximately 6 h after infection, the bacteria lyse the host cell and infect neighboring eucaryotic cells, forming "plaques" in epithelial cell monolayers.

The genetics of *Shigella* invasion are complex and have been reviewed elsewhere (92, 94, 150). The genes required for *Shigella* invasion are encoded on a 120- to 140-MDa plasmid which is required for virulence (97, 213, 217, 231, 267). (Also, at least three chromosomal regions are needed for virulence, but these loci do not appear to be required for invasion and are probably involved in survival once these organisms have entered host tissues.) A large fragment of the *Shigella flexneri* 140-MDa plasmid can be cloned and confers the invasive phenotype (149). Work by several groups indicates that there are five virulence-associated regions clustered within approximately 30 kilobases on this plasmid which are needed for bacterial entry into eucaryotic cells (12, 13, 36, 92, 216, 217). Encoded within this region are polypeptides which elicit host antibody production during *Shigella* infections; the corresponding genes have been named "invasion plasmid antigen" genes, or *ipaB*, *ipaC*, and *ipaD*. The products of these genes are expressed on the bacterial surface and probably form part of a complex constituting the *Shigella* invasion determinant, as products from these genes can bind to eucaryotic cell surfaces and are necessary for invasion. Approximately 43 kilobase pairs distant to the Ipa gene cluster is another region, *virF*, which is also required for *Shigella* invasion (211). Recent data indicate that this region encodes a 30-kDa positive regulator which controls transcriptional expression of the *ipaB*, *ipaC*, and *ipaD* genes as well as another genetic cluster, *virG*; the *virF* gene product is therefore essential for *Shigella* invasion (212, 216).

The *virG* gene is a 4-kilobase plasmid-encoded locus which is not needed for bacterial entry into epithelial cells or intracellular replication (145). It is separated by approximately 30 kilobase pairs from the Ipa region and is about 106 kilobase pairs distant from *virF*. The *virG* gene is essential for the spread of intracellular bacteria to adjacent cells in tissue culture models. Strains which bear mutations in this gene enter cells normally, but remain localized in the cell without moving to neighboring cells.

Salmonella

Invasion of the gastrointestinal mucosa is an essential step required for *Salmonella typhimurium* pathogenesis (81), and strains unable to invade animal cells are avirulent (82). However, in contrast to *Shigella* species, most *Salmonella* species proceed through the surface intestinal epithelial cells into deeper tissue and often enter reticuloendothelial cells. A comprehensive electron microscopy study of *Salmonella* intestinal epithelium penetration was published by Takeuchi (251). As *S. typhimurium* bacteria came into close proximity to the brush border, the epithelial microvilli began to degenerate. The bacteria entered into the epithelial cells and resided within membrane-bound cavities, similar to those seen following *Shigella* entry. However, unlike the *Shigella* species, *Salmonella* species remain within the membrane-bound inclusion. A comparable chain of events for *Salmo-*

nella invasion has been observed in cultured animal cells (65, 67). Although each invading organism enters into a separate vacuole, these coalesce and at later times most intracellular organisms are found within a single large intracellular vacuole. Both *Salmonella* and *Shigella* species require functional host microfilaments for entry (65, 66), and the invading bacteria are surrounded by polymerized actin during internalization (46; B. B. Finlay, J. Fry, E. P. Rock, and S. Falkow, J. Cell Sci., in press). Using murine ileal loops infected with *S. typhi*, Kohbata et al. reported that ileal M cells, a type of intestinal epithelial cell found in Peyer's patches, may be the site of primary host cell entry for *S. typhi* (126). Indeed, most *Salmonella* organisms favor the cells of the terminal ileum, where they presumably enter both epithelial cells and the specialized M cells. This is in contrast to *Shigella* spp., which appear to enter columnar intestinal epithelial cells, the predominant cell type lining the intestine. *Salmonella* entry and intracellular replication do not require endosome acidification (65).

After entry into epithelial cells, *Salmonella* spp. continue through the cell and penetrate (transcytose) to the opposite surface of the epithelial cell (67, 251). A polarized epithelial model has been developed, allowing the study of transcytosis in vitro (67). In this system, *Salmonella* spp. preferentially bound to the apical (top) surface of polarized epithelial cells, caused loss of apical epithelial microvilli, and also caused disruptions in the epithelial tight junctions. The minimum time required to transcytose to the opposite surface was 4 h.

Salmonella adherence to and invasion of eucaryotic cells is an active event requiring bacterial protein and ribonucleic acid (RNA) syntheses, but not DNA replication (67a). This finding underscores the observation that neither *Salmonella* nor *Shigella* species grown in broth bind to any significant degree to eucaryotic cells. It has recently been shown that *S. cholerae-suis* and *S. typhimurium* synthesize several new polypeptides required for adherence and invasion following their interaction with epithelial cell surfaces (67a). The stimulus for synthesis of these novel proteins appears to be a structure(s) on the epithelial cell surface which is sensitive to trypsin and neuraminidase. Inhibition of eucaryotic protein synthesis does not affect *Salmonella* invasion (67a).

The genetics of *Salmonella* invasion are not as well defined as that of *Shigella* invasion. Many highly pathogenic *Salmonella* species (with the notable exception of *S. typhi*) harbor a plasmid which is essential for virulence (86, 87, 100, 117, 120, 254), although this extrachromosomal element is not needed by *Salmonella* spp. to enter epithelial cells. Plasmid-cured strains can be found in the reticuloendothelial system within experimental animals (89), but the plasmid is required for prolonged survival within the host and cured strains are cleared rapidly from the spleen. Six classes of Tn*phoA* mutants of *S. cholerae-suis* were recently described which are unable to enter epithelial cells (68). Two of these classes caused defects in core or O-side-chain lipopolysaccharide molecules; the effects of the other four are unknown, although one class of mutants did not synthesize the induced invasion proteins discussed above. Mutants belonging to all six classes were also unable to adhere to eucaryotic cells, yet none of the insertions were in the genes encoding type 1 pili or mannose-resistant hemagglutinin (98). Four of the six mutant classes were avirulent in orally challenged mice. Also, we have identified two Tn*10* mutants of *S. typhimurium* which do not enter epithelial cells or macrophages and are avirulent in mice (B. B. Finlay, S. Falkow, and F. Heffron, manuscript in preparation).

Salmonella spp. have nutritional requirements which change once organisms are inside a host cell; these modified requirements are being defined. Mutations which affect aromatic amino acid (*aro*) and purine (*pur*) biosyntheses cause these strains to be attenuated and have decreased virulence because these nutrients are not available from the animal host (31, 153, 242). These strains can still penetrate cells and reach the liver and spleen, where they may persist for a few weeks, similar to cured *Salmonella* strains. *S. typhimurium* strains lacking adenylate cyclase and the cyclic adenosine 3',5'-monophosphate receptor protein are avirulent in mice, probably because the many catabolic operons under cyclic adenosine 3',5'-monophosphate control are no longer activated (51).

During the infection cycle, *Salmonella* spp. may be internalized by macrophages, within which they can survive. Fields et al. (63) identified 83 Tn*10* mutants of *S. typhimurium* which exhibited decreased survival rates in macrophages. These mutants represented several distinct phenotypes and were avirulent in mice. Some mutants were auxotrophic, some were hypersensitive to serum, some had altered response to oxidative stress, and others were nonmotile. Mutations in *phoP*, a positive regulator gene of alkaline phosphatase and several other gene products, cause increased sensitivity of *S. typhimurium* to the bactericidal cationic proteins found in macrophages (62a). As these mutants are characterized further, we will learn more about the factors involved in intracellular survival within phagocytic cells.

Yersinia

Because the pathologies of *Salmonella* and *Yersinia* infections are so similar, it was expected that these bacteria would have similar invasive mechanisms quite distinct from those of *Shigella* species. It has since become obvious that, beyond the similarity of being invasive, the bacterial genes and mechanisms necessary for entry are markedly different for all three genera.

Yersinia pseudotuberculosis and *Yersinia enterocolitica* infections proceed by routes similar to those for *Salmonella* species. These organisms are transmitted by the fecal-oral route and proceed to the small bowel, where they are taken up in Peyer's patches (263). Whether these bacteria utilize M cells for uptake remains to be determined. Once through the intestinal epithelium, these organisms are also internalized by cells of the reticuloendothelial system; they then migrate to the lymph nodes and spleen.

The initial events of *Yersinia* entry into cultured cells suggest that these bacteria are internalized by host cell mechanisms which appear similar to those described for *Salmonella* and *Shigella* species. (For a recent review on *Yersinia* invasion, see reference 164.) Internalized *Yersinia* spp. are surrounded by a membrane-bound inclusion, and internalization can be blocked by microfilament inhibitors (34, 65, 66). As observed for *Salmonella* and *Shigella* species, endosome acidification is not required for *Yersinia* entry; like the salmonellae, *Yersinia* cells remain within vacuoles (65). However, intracellular replication of *Yersinia* species is much slower than for *Salmonella* or *Shigella* organisms and can only be detected in a few eucaryotic cell lines (65). *Yersinia* spp. enter singly, enclosed within a vacuole, but these vacuoles do not appear to coalesce as observed with *Salmonella* spp. Escape of intracellular *Yersinia* spp. from the eucaryotic cell remains poorly defined.

In contrast to the other invasive enteric bacteria, *Yersinia* species grown in the laboratory immediately adhere to eucaryotic cells, even at 4°C, and are internalized within minutes (33, 110). Ultraviolet light-inactivated or Formalin-fixed *Y. enterocolitica* (188, 265), or those treated with bacterial RNA and protein synthesis inhibitors (B. Finlay, unpublished observations), are readily taken up by eucaryotic cells. Thus, unlike *Salmonella* invasion, de novo bacterial biosynthesis is not required for *Yersinia* invasion. Rather, the bacterial components required for *Yersinia* invasion are already present on the surface of the organism and are synthesized constitutively.

Yersinia species are thought to spend much of their time within phagocytic cells such as macrophages (263). As already described for salmonellae, novel mechanisms are required for life within this niche. *Yersinia* spp. reside within vacuoles in professional phagocytic cells and appear to multiply within these cells (43, 245, 246, 263). Phagolysosomal formation and degranulation appear to occur normally inside cells infected with *Y. pestis*, but the oxidative burst is decreased (43). Although *Yersinia* species also harbor a virulence plasmid (49, 198), this plasmid is not required for invasion of any cell type or for intracellular survival. The plasmid is needed for intracellular multiplication (43).

In contrast to *Salmonella* and *Shigella* spp., the genetics of *Yersinia* cell entry have been relatively tractable and have focused on discrete chromosomal genetic loci (reviewed in detail elsewhere [164]). The *inv* (for invasion) gene of *Y. pseudotuberculosis* is a 3.2-kilobase-pair region on the chromosome which encodes a single, large polypeptide (invasin) that is exposed on the bacterial surface (109, 110). When this gene is expressed in a noninvasive *E. coli* genetic background, these recombinant bacteria are able to enter cultured animal cells. *Y. pseudotuberculosis inv* mutants are unable to adhere to or enter tissue culture cells, indicating that adherence and invasion are synonymous features of the invasin protein. A homologous gene is present in *Y. enterocolitica*, and *E. coli* recombinants harboring this gene also invade tissue culture cells (162).

Another invasion gene has been identified in *Y. enterocolitica* which is different from the *inv* genes. *ail* (for attachment invasion locus) is a small (650-base-pair) locus on the *Y. enterocolitica* chromosome which, when introduced into noninvasive *E. coli*, transforms the organisms into strongly adherent and invasive bacteria (162). *ail* encodes a 15-kDa membrane protein which is efficiently expressed in *E. coli*.

The roles that the *inv* and *ail* loci play in vivo for *Yersinia* invasion are still under investigation. Epidemiologically, there is an excellent correlation between the presence of DNA homologous to these genes and the presence of significant *Y. enterocolitica*-related clinical disease (163, 164). Although all *Y. enterocolitica* and most other *Yersinia* species have sequences homologous to an *inv* DNA probe, five hybridization patterns have been observed. Clinical isolates having either one of two of these patterns are invasive for cultured cells and are associated with disease outbreaks, while bacteria having any of the other three patterns are noninvasive for cultured cells and of doubtful clinical relevance. All *Y. pseudotuberculosis* strains tested have only one type of hybridization pattern to the *inv* probe and are invasive. The presence of *ail*-specific DNA sequences is an even better indicator of an invasive phenotype. Only the pathogenic *Yersinia* species have homology to the *ail* probe. Moreover, *Y. enterocolitica* isolated from disease possess homologous sequences, while those not associated with disease or not capable of entering cultured cells are completely devoid of *ail* sequences. Thus, this invasion-specific

probe serves as an excellent means to detect pathogenic *Yersinia* spp.

Salmonella, *Shigella*, and *Yersinia* species appear to exploit and utilize common eucaryotic cell functions to enter the intracellular environment initially. The events subsequent to entry indicate that these three genera behave quite differently with respect to the nature and complexity of the bacterial products required for intracellular survival and multiplication (Table 1). Species from all three groups require active microfilament participation and do not require endosome acidification, yet there does not appear to be any relatedness at the nucleotide or amino acid sequence level among the various genes involved in invasion. Presumably this reflects the fact that each of these invasive microorganisms has selected a distinct strategy following internalization. *Shigella* species escape the vacuole immediately following entry before subsequent intracellular replication ensues. In contrast, *Salmonella* and *Yersinia* species remain within membrane-bound inclusions provided by the host. *Salmonella* species replicate intracellularly within the confines of a large vacuole formed by the coalescence of many smaller vacuoles, each initially containing a single bacterium. Perhaps this step is driven by the bacteria as a prerequisite for replication. *Yersinia* species multiply slowly, if at all, inside epithelial cells. Metabolically inactive *Yersinia* species can adhere to and be internalized by eucaryotic cells, but *Salmonella* and *Shigella* species must be viable and, at least in the case of *Salmonella* species, synthesizing RNA and proteins. The reasons for these differences remain unclear. One could speculate that the behavior of each organism within an epithelial cell provides some insight into its overall pathogenic strategy. *Shigella* spp. immediately begin a replicative event that is lethal to the cell. Both *Salmonella* and *Yersinia* species replicate to a lesser extent or not at all. Perhaps they utilize this event to prepare themselves metabolically for the hazards that await them after they leave the safety of the superficial cellular layers of the host. After all, the major site of replication in the host for *Salmonella* and *Yersinia* species is not the epithelial cell as it is for the shigellae. Rather, it is the reticuloendothelial system that is the key to the success of both the salmonellae and the yersiniae. It is likely that this is where we must look to find many other essential features of the invasive phenotype and the intracellular lifestyle. Despite their differences, the organisms belonging to these three groups provide yet another example of a common theme in pathogenesis: to penetrate the intact cellular layers of the host and eventually replicate.

Receptors

The identities of the eucaryotic receptors which invading organisms use to enter the host cell are not well defined. The concept of receptor specificity applies to both adherence and invasion. The specificity of receptors presumably helps to determine the type of cell and, hence, intracellular environment that the organism enters and, ultimately, the disease manifestations produced. Some organisms can enter eucaryotic cells of extremely diverse nature. For example, both *Salmonella* and *Yersinia* spp. can enter embryonic *Drosophila* cell lines as efficiently as human intestinal cells (B. Finlay and J. Bliska, unpublished results), indicating a common receptor(s) and internalization mechanism. However, other organisms enter only a specific cell type or have a limited host cell range which is determined by the presence of a specific receptor.

Recently, the receptor for invasin from *Y. pseudotuberculosis* has been identified as a eucaryotic surface protein belonging to a superfamily of structurally related receptors known as integrins (R. Isberg, personal communication). These proteins consist of heterodimers, with each subunit spanning the eucaryotic cell membrane (209). Integrins perform a variety of functions necessary for eucaryotic cell attachment to extracellular matrices, phagocytosis, and cell-cell adhesion. Integrins bind several proteins present in extracellular matrices and blood, including fibronectin, laminin, collagens, and vitronectin, which contain a conserved sequence, Arg-Gly-Asp (RGD) (208, 209). The RGD sequence is responsible for binding these extracellular eucaryotic molecules to their integrin receptor, although other factors also contribute to determine the integrin-binding specificity. (As mentioned previously, *Treponema pallidum* also binds to the RGD sequence of fibronectin.) In addition, integrins interact with talin, a cytoskeletal protein associated with the intracellular actin filament network. Although the *Y. pseudotuberculosis* invasin does not contain an RGD sequence, attachment to an integrin may facilitate internalization of this bacteria by utilizing the host actin filaments via talin.

Also belonging to the family of integrins is the Mac-1 (or CR3) protein, the cell receptor for C3bi which mediates opsonic phagocytosis. Interestingly, *Legionella pneumophila*, *Mycobacterium tuberculosis*, and *Leishmania donovani* all use complement receptors to mediate their uptake into phagocytic cells (23, 103, 185, 186), yet *Legionella pneumophila* and *Leishmania donovani* follow different pathways through the cell. It has recently been demonstrated that a major surface glycoprotein (gp63) of *Leishmania* sp. contains an RGD sequence which binds to the CR3 receptor (210). Thus, parasites have devised methods to mimic host proteins to take advantage of preexisting receptors and facilitate their uptake.

The ability to utilize integrins may not be limited to intracellular bacteria. Recently, the nucleotide sequence of the filamentous hemagglutinin from *Bordetella pertussis* has been determined (D. A. Relman, M. Domenighini, E. Tuomanen, R. Rappuoli, and S. Falkow, Proc. Natl. Acad. Sci. USA, in press). This large surface protein is thought to function as an adhesin for *Bordetella pertussis*, enabling this organism to adhere to human ciliated respiratory epithelial cells. The predicted amino acid sequence of filamentous hemagglutinin includes an RGD sequence which is predicted to be surface exposed. Seven of nine residues at this site share homology with the RGD site of fibronectin. Perhaps this organism uses this filamentous hemagglutinin sequence to bind to an integrin molecule, enabling the bacterium to colonize the respiratory tract.

Life within Phagocytic Cells

Microbial life within phagocytic cells requires many adaptations (171). Intracellular organisms must be able to avoid or resist the many antibacterial agents which exist in vacuoles in these cells (259). Three mechanisms are postulated for survival within phagolysosomes (171). The first is to avoid entering the macrophage by a pathway which leads to fusion of the lysosome with the vacuole containing the bacterium. It is difficult to discriminate between organisms that enter via this route and those that actually inhibit endosome acidification and phagolysosomal fusion, the second route. *Legionella pneumophila* enters macrophages by a process termed coiling phagocytosis (105). These organisms

inhibit both endosome acidification and lysosome-phago-some fusion events, presumably making the environment less harsh for the intracellular organisms (104, 106). Other intracellular parasites which inhibit host endosome acidification include *Toxoplasma gondii* (228) and *Nocardia aster-oides* (22). Inactivated *Legionella* spp. enter by the same mechanism, but do not inhibit these events. The third mechanism is to withstand or neutralize the antibacterial agents delivered by phagosome-lysosome fusion. As mentioned above, both *Salmonella* and *Yersinia* species live in such an environment, as does *Coxiella burnetii* (4, 35).

ESTABLISHMENT

Success for an infecting microorganism is measured by its capacity to multiply sufficiently to establish itself within the host or to reach sufficient numbers to ensure transmission to another susceptible individual. As this occurs, the bacteria may secrete toxins which cause tissue damage. Also, the infecting microorganisms are exposed to the nonspecific and specific immune systems of the host, and these must be avoided, subverted, or nullified.

Role of Cytotoxins

Although some potent bacterial toxins are probably the best-characterized virulence determinants, their actual roles in microbial pathogenicity have not been clarified (122, 141). Bacteria which cause diseases as a direct result of toxin secretion are usually avirulent when the toxin gene(s) is removed. However, these modified bacteria are not necessarily devoid of their infectivity. For example, non-toxigenic *Corynebacterium diphtheriae* can still infect humans and can occasionally cause symptoms of disease, although toxigenic *Corynebacterium diphtheriae* probably colonize their host more efficiently. We propose that diphtheria toxin contributes to the pathogenesis of infection by modifying the microenvironment of the nasopharynx so that *Corynebacte-rium diphtheriae* can outgrow local competitors, such as the streptococci. This principle is similar to that reported by Loeffler almost a century ago that the diphtheria bacillus grows better on coagulated serum than does the average common inhabitant of the human throat. The local effects of diphtheria toxin may create a layer of dead cells which serves as a medium for bacterial growth. This strategy for successful competition would have an occasional consequence for the host which could be unfortunate, but presumably not so devastating as to destroy the host-parasite relationship. It is not understood how most of the potent bacterial toxins associated with human and animal diseases participate in the natural ecology of the bacteria that produce them. Human disease as a consequence of a traumatic war wound or accidental ingestion of spoiled food is an individual disaster, but not necessarily very revealing about the actual role of *Clostridium perfringens* toxin in the pathogenesis of infection or its role, if any, in the colonization of this species in the gastrointestinal tract of animals. For the toxigenic microorganisms, such as *Corynebacterium diphtheriae* (20, 157), *P. aeruginosa* (196, 276), and *Bordetella pertussis* (268), which regularly infect susceptible humans, we understand clearly the biochemical basis for toxigenicity but have little insight into their biological roles in the life of the microorganism.

Fortunately, molecular cloning techniques coupled with the appropriate infection models can lead to the elucidation of the roles of some toxins in the pathogenesis of infection.

In this way, it has been demonstrated recently that Shiga toxin has no effect on the invasion of cells or the intracellular growth rate or even on the rapid killing of invaded host cells (47, 214). Rather, the production of Shiga toxin by an invading strain is correlated with colonic vascular damage, which accounts for the bloody stools, intestinal ischemia, and an increase in polymorphonuclear cells within the intestinal compartment. Thus, while Shiga toxin is not an essential determinant of pathogenicity, it is clearly an important virulence factor which influences the severity of bacillary dysentery. The production of Shiga toxin by noninvasive shigellae permits an even closer look at some of the potential contributions of Shiga toxin to the pathogenesis of disease.

Other less potent bacterial toxins probably play more subtle but no less important roles in bacterial infection. There is a plethora of bacterial toxins reported in the literature that are well characterized biochemically, yet their roles in pathogenicity have not been well defined. These toxins and their accessory proteins often exhibit homology between bacterial species, as observed with *E. coli* hemolysin and *Bordetella pertussis* adenylate cyclase and leukotoxin (L. Gray, personal communication). One of the challenges of studying microbial pathogenicity is to define the role of these toxins in pathogenesis. Sometimes tissue damage may be required to allow bacteria to penetrate into deeper tissue or pass through a host epithelial or endothelial barrier. Toxin elaboration may also inhibit the immune response of the host or, perhaps in the case of enterotoxins, flush away competing bacterial neighbors (39). Toxins are often just one of several virulence factors produced by microbial pathogens (136), and although toxins may represent the principal determinant of virulence and the cause of disease, they may not be the principal determinant of infectivity.

Avoidance of Host Immune Systems

The highly efficient host immune system is made up of many components, each of which is capable of destroying bacteria. Microbial pathogens have evolved a number of ways to escape this system (83).

Antiphagocytic activity. A fundamental requirement for many pathogenic bacteria is to escape phagocytosis by macrophages and polymorphonuclear phagocytes. We presume that the capacity to avoid phagocytosis was also an early necessity for a number of microorganisms. Bacteria must have been prey to phagocytic amoebae at an early time in their evolution. Some bacteria, such as *Legionella* spp., presumably learned to utilize the free-living amoebae as part of their life cycle. Now one sees *Legionella* spp. using similar mechanisms to outwit human macrophages (103).

The most common means utilized by bacteria to avoid phagocytosis is an antiphagocytic capsule (101, 112, 167, 172, 229, 260). The significance of the capsule can hardly be overemphasized. All of the principal pathogens which cause pneumonia and meningitis, including *Haemophilus influenzae*, *Neisseria meningitidis*, *E. coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and group B streptococci, have polysaccharide capsules on their surface. Nonencapsulated derivatives of these organisms are usually avirulent. Although the chemical composition of these capsules can vary significantly between strains and species, most capsules are composed of polymers of repeated sugar residues. However, only a few types of capsules are commonly associated with disease. *H. influenzae* isolates can produce one of six different types of polysaccharide capsules, yet organisms

expressing type b capsules are the predominant isolate from serious infections (144, 172). Capsules from disease-causing bacterial pathogens prevent complement deposition on the bacterial surface, while capsules from nonvirulent strains are less efficient at preventing this deposition (115). Capsules are only weakly immunogenic and mask more immunogenic underlying bacterial surface structures and would directly activate complement. Thus, the capsule prevents opsonization of the organism, conferring resistance to phagocytosis.

The group A streptococcal M protein is an example of an alternative bacterial product used by the organism to escape opsonization and phagocytosis. This surface protein confers resistance to phagocytosis by preventing opsonization by complement (135, 272). Avirulent organisms lacking the M protein are readily opsonized by complement which has been activated by the alternate pathway (21, 190). The resistance to opsonization and phagocytosis is due, in part, to the ability of the M protein to bind fibrinogen and its breakdown product fibrin (270–272). Binding these molecules to the M protein sterically hinders complement access to the bacterial surface and prevents opsonization. Just how the pathogenic bacteria bind host proteins to "confuse" the host defense system is not known, but it may be a more common theme than realized. This possibility again emphasizes the need to develop experimental procedures that permit us to analyze microorganisms in their natural habitat rather than in the laboratory setting.

Antigenic variation. Another method by which microbes avoid host immune responses is to vary surface antigens (160, 224). As with most aspects of microbial pathogenesis, several mechanisms are used. *Neisseria gonorrhoeae* is a master chameleon, possessing at least two mechanisms for alteration of surface antigens. The PII protein is a gonococcal surface protein which can alter colony opacity. Most *Neisseria gonorrhoeae* express several different PII proteins at any given time, and a single strain can potentially express up to seven different pII proteins (223, 249, 266). The genetic control of each PII gene appears unrelated to other PII genes, which results in the presentation of many different combinations (239, 240). The regulation of PII gene expression depends on the repeating five-nucleotide CTCTT, which is located within the PII leader sequence (38). Variation in the number of repeats of this pentamer (through recombination or infidelity during DNA replication) will vary the reading frame of the downstream PII gene, and a functional PII protein will be synthesized only when the correct reading frame is translated. (Messenger RNA transcription from "on" and "off" PII genes is constitutive.)

Another example of antigenic variation in the gonococcus is found with the pilin genes (19, 160, 224, 250). In *Neisseria gonorrhoeae*, there is usually only one complete pilin gene that is expressed, although there are many incomplete pilin gene sequences that are silent in the gonococcus (159, 161). These incomplete pilin genes have many differences in addition to several conserved regions. The gonococcus can undergo "gene conversion" by placing one of these incomplete sequences into the expression site, displacing the previous pilin gene, and synthesizing a new, antigenically distinct pilin molecule (88, 91, 159). Alternatively, the gonococcus may acquire pilin sequences from other lysed bacteria via a transformation event (224). Homologous recombination of a transformed pilin gene into the expression site would also generate a new pilin molecule. Whatever the mechanism used, periodical switching of pilin genes can alter the antigenicity of the gonococcus pili.

As mentioned above, gonococcal pili belong to the *N*-

methylphenylalanine pilin group. Other bacteria which make *N*-methylphenylalanine pili express different pilin sequences between strains. For example, each strain of *P. aeruginosa* expressed only one pilin type from a single pilin gene (183). However, there are several antigenically different pilin types expressed by various strains of *P. aeruginosa* (184, 218). There are also two types of *Moraxella bovis* pili, both of which can be expressed in this organism (146, 147). *Bacteroides nodosus*, the causative agent of sheep foot rot, also has several antigenically different types of pili (6). Thus, the pilin genes of the *N*-methylphenylalanine group can vary significantly between strains of these species, thereby preventing the host from making "species-specific" pilin antibodies. Despite these similarities, these organisms differ in their mechanisms of antigenic variation. Variation between pilus types of *Moraxella bovis* is the consequence of a genetic inversion more closely akin to the well-known phase variation of *Salmonella* flagella genes and type 1 pili. It is instructive to see that, by the use of genetic recombination mechanisms, microorganisms can use alternative means to achieve the same final pathogenic theme.

It is thought that *Borrelia* sp., the cause of relapsing fever, uses a mechanism similar to *Neisseria gonorrhoeae* pilin variation to express different variable major proteins and thereby undergo antigenic variation (10, 11, 156, 160, 224, 243). These organisms possess silent copies of the genes encoding variable surface proteins on a linear plasmid and the expression site for these proteins on another plasmid. Silent copies can be moved to the expression locus, leading to the expression of new surface proteins.

A major surface molecule of group A streptococci is the M protein. There are at least 75 different serotypes of M-protein molecules. These molecules are encoded by genes rich in tandemly repeated sequences, and the resulting proteins have a region of repeated seven-residue periodicity (69, 70, 102, 166). It is thought that homologous recombination of DNA from these regions generates the diversity in size and sequence observed for this protein.

IgA proteases. Several organisms produce enzymes which are capable of cleaving secretory immunoglobulin A (IgA) antibodies. These IgA proteases are found in *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *H. influenzae*, *Streptococcus pneumoniae*, *Streptococcus sanguis*, and other species that infect the oral cavity (123, 181, 193). These organisms often colonize mucosal surfaces, where the predominant isotype of antibody is IgA. Cleavage of secretory IgA would presumably enhance the ability of an organism to survive on mucosal surfaces. The IgA proteases secreted by these organisms are specific for human IgA, usually the IgA1 isotype, and cleave the IgA1 heavy chain into two fragments, Fab and Fc (193). The peptide sequences which these enzymes hydrolyze vary between organisms, but occur within a small region in the hinge region of the heavy chain. (This region is missing in human IgA2, and thus this molecule is not cleaved.) The IgA1 protease genes from *Neisseria gonorrhoeae* (127, 194) and *H. influenzae* (29) have been cloned and expressed in *E. coli*.

Nearly all of the organisms which produce IgA proteases also require a capsule for virulence. (An exception is the gonococcus.) The production of a capsule (to avoid opsonization) and of IgA proteases (to cleave secretory antibodies) are two features common to the predominant pathogens which cause meningitis. These organisms are usually found on mucosal surfaces before they enter deeper tissues and penetrate the blood-brain barrier. The possession of

these two defense mechanisms presumably enhances bacterial persistence on host mucosal surfaces.

Serum resistance. Another mechanism used by pathogens to avoid host defense mechanisms is to prevent lysis by complement, a process known as serum resistance (48, 113–115, 260). Pathogenic organisms evade complement lysis by a variety of means. *Salmonella* species contain O antigens in their lipopolysaccharide (204) and are more resistant to complement than isogenic strains lacking lipopolysaccharide (rough strains) (85). The protection afforded by O antigens is due to steric hindrance of the C5b-9 complement complex, inhibiting its access to hydrophobic domains in the bacterial outer membrane (114). Alternatively, various O-antigen polysaccharides can activate the alternate complement pathway to various degrees, and the degree of this activation is inversely proportional to virulence (220). In contrast, *Campylobacter fetus* inhibits the complement cascade by limiting C3 deposition on the bacterial surface, probably due to the presence of high-molecular-weight capsular proteins (24). The sialic acid-containing capsules of *E. coli* K1 and *Neisseria meningitidis* group B also confer serum resistance by preventing efficient complement activation. *Neisseria gonorrhoeae* is able to resist complement activation by another mechanism. The C5b-9 complex interacts with the bacterial surface, but forms an aberrant configuration in the outer membrane (116). Finally, as discussed above, another way to avoid the host defenses is to enter and exist within host cells as an intracellular pathogen.

DISSEMINATION WITHIN THE HOST

Once a pathogenic organism has entered into a eucaryotic cell, it can often pass through that cell to enter deeper tissue or the blood. Passage through epithelial barriers in vitro has been described for *Neisseria* and *Salmonella* species (67, 154). As mentioned above, the genes required for *Salmonella cholerae-suis* adherence and invasion are also required for passage through an epithelial barrier (68). As more model systems are developed, scientists can begin to address both the bacterial and the host cell factors required for passage through and escape from eucaryotic cells. Another possible mechanism of bacterial release from a cell is lysis of the host cell. This lytic activity may involve the action of specific cytotoxins. Alternatively, large amounts of bacterial intracellular multiplication could stress the host cell to the extent that it would cause the eucaryotic cell to burst and release the intracellular organisms. Presumably, this mechanism would also lead to localized tissue damage.

Once through host epithelial barriers, bacteria can potentially disseminate throughout the host. As mentioned above, some organisms may remain within phagocytic cells, by which they are transported to lymph nodes. From the lymph collecting system, bacteria may enter the blood, gaining quick access to most of the host. Blood-borne pathogens may use this method of transport to travel to the blood-brain barrier or other endothelial barriers.

Some bacterial pathogens penetrate epithelial and endothelial barriers and enter deeper tissue by passing between the eucaryotic cells forming these barriers. *Treponema pallidum*, the causative agent of syphilis, can penetrate endothelial monolayer barriers by passing through intercellular junctions between endothelial cells in an in vitro system (258). These spirochetes may leave the bloodstream by passing between endothelial cells of the vascular system within a host. Preliminary evidence indicates that *Borrelia*

species may use a similar pathway (M. A. Lovett, personal communication). *H. influenzae* can also disrupt epithelial tight junctions and pass between epithelial cells in a human nasopharyngeal organ culture model (62).

Organisms capable of disseminating via the bloodstream usually have developed special mechanisms to acquire iron. (This is not a problem for intracellular organisms, as iron levels within cells are much higher.) Most iron within mammalian hosts is tightly complexed with iron-binding proteins such as transferrin, lactoferrin, and ferritin. The lack of free iron in hosts serves as an antimicrobial device, since bacteria also require iron (7, 64, 187). It is known that increased levels of iron may lead to increased numbers of certain infections (8, 25). To circumvent this lack of free iron, pathogenic organisms produce siderophores which are capable of removing iron from host proteins, freeing it for bacterial use after uptake by an iron transport system. As an alternative mechanism, *Neisseria meningitidis* has human transferrin and lactoferrin receptors on its surface which bind and internalize the host's molecules and their bound iron, satisfying the iron requirement of the bacterium (221, 222).

ROLE OF THE HOST

As discussed throughout this review, many bacterial factors contribute to the likelihood that a host-pathogen interaction will result in disease. There are also a multitude of host factors which contribute to determination of the outcome of an infection (167). Although the contribution of host factors to disease is extremely complex and more difficult to study than bacterial virulence factors, this aspect must be considered when studying microbial pathogenesis. The host factors affecting susceptibility to microbial and parasitic infection are beyond the scope of this article, and in this section we only touch briefly upon a few areas of recent or recurrent research interest.

The genetic constitution of a host often contributes to the susceptibility of an individual to an infection and resulting disease, as most host functions are controlled genetically. This aspect has been best studied in animal hosts when inbred animals are available. It is known that several genetic factors in mice (*Ity*, *Lsh*, and *xid*) contribute to the susceptibility of these animals to *Salmonella* infections (99). The *Ity* gene also controls resistance to *Leishmania donovani* (192), *Mycobacterium bovis* (230), and possibly *Mycobacterium leprae* (30). Susceptible and resistant lines have been bred, and the roles of these host factors in bacterial infection are being characterized (138). It is also known that humans with sickle cell anemia are more susceptible to extraintestinal *Salmonella* infections than normal individuals (207). In contrast, the sickle cell trait confers resistance to malarial infections, as sickle erythrocytes are less readily parasitized than normal cells (143). Malarial resistance in these individuals contributes to the persistence of the sickle cell trait in Africa, since it bestows a selective advantage on individuals in these regions where malaria is endemic (119).

The host genetic composition operates at the level of the immune response. Recently, several diseases have been linked to the major histocompatibility loci (HLA genes) (279). The products of this region are involved in foreign antigen presentation to the immune system. Cross-reactivity between bacterial antigens and self can lead to autoimmune diseases. Postinfectious reactive arthritis (Reiter's syndrome) is one such example (3, 61, 121). This arthritis is closely associated with the HLA antigen B27 and is probably

due to cross-reactivity of bacterial antigens with host molecules. Most humans with ankylosing spondylitis are also of the HLA-B27 type. It appears that some antigens of gut-associated bacteria such as *Klebsiella* spp. are cross-reactive with HLA-B27 molecules, and this cross-reactivity contributes to the autoimmune nature of the disease (179). Rheumatic fever is another disease which is probably the result of cross-reactivity between host molecules and a bacterial antigen (the streptococcus M protein) (reviewed in reference 61). The utility of transgenic animals to examine some of these aspects of disease and how microbial pathogenic traits affect the host will surely become an exciting avenue of research in the near future.

Since the immune system is a major line of defense against infection, the immune status of an individual contributes significantly to the outcome of bacterium-host interactions. Immunosuppressed and immunocompromised individuals are much more susceptible to infections by virulent organisms and often have a more severe form of disease. These individuals are also more likely to be infected with opportunistic organisms usually considered "nonpathogenic" in a healthy population. Humans with the acquired immunodeficiency syndrome are often infected with *Pneumocystis carinii*, yet this organism rarely causes disease in normal individuals. Individuals with human immunodeficiency virus infection are also more susceptible to symptomatic *Salmonella* bacteremia caused by species usually associated with gastrointestinal symptoms (237). Presumably, the compromised immune system in these individuals is unable to contain these organisms and the resulting infection. One supposes that the newly recognized capacity to reconstitute the human immune system in experimental animals (152) will finally permit us to investigate human-specific pathogens within an immunologically defined experimental model.

During infancy and old age, the immune system is less effective (79, 191). Thus, diseases which affect infants are often less severe or not present in older children or young adults. Infants and the elderly also have poorer mechanical defenses against infection, and this is reflected by the higher incidence of pulmonary infections in these populations.

Various external stresses on the host also contribute to the outcome of an infection. Malnourished individuals suffer from impaired immune responses (42, 201). Mental stress may also contribute to disease susceptibility, presumably because of hormonal consequences. Hospitalization is also associated with altered host factors and resultant disease susceptibility. It is known that individuals undergoing heart transplants and other major operations shed fibronectin from their oral and upper respiratory mucosal surfaces (274). This alters the host environment at these surfaces and leads to changes in bacterial adherence: *Staphylococcus* and *Streptococcus* spp. bind to fibronectin on these surfaces. Conversely, fibronectin prevents *E. coli* and *P. aeruginosa* from colonizing these same surfaces. In surgical patients, the number of respiratory infections caused by enteric organisms is relatively increased, reflecting this change in fibronectin distribution (1, 238, 274, 277, 278).

REGULATION OF PATHOGENIC MECHANISMS

As mentioned previously, most pathogenic bacteria lead a schizophrenic existence, spending time both within and outside hosts or within two different types of host (e.g., arthropod-borne pathogens). Pathogens are also continually moving through different environments once inside a host. It is difficult to imagine that organisms would synthesize products required specifically for life inside a host while dwelling outside. There is a growing body of evidence showing that bacteria are constantly sensing their environment and adjusting to it (235; J. Miller et al., Science, in press). Thus, pathogens go through several transitions as they move throughout the host. There are many examples of bacteria adjusting to the presence of new or altered levels of nutrients by regulating various gene products (84, 241). However, only recently have we begun to identify and characterize the regulatory mechanisms associated with expression of virulence factors in the host environment. Many of the mechanisms used to control virulence factors share features with other bacterial regulatory systems.

The virulence determinants of *Bordetella pertussis* are all regulated by a single genetic locus, *vir* (269). Products of this locus function as a positive inducer of many virulence genes, including those which encode filamentous hemagglutinin, pertussis toxin, adenylate cyclase, hemolysin, fimbria subunits, and dermonecrotic factor. Inactivation of this locus results in the lack of expression of at least 20 gene products. *vir*-controlled gene products are negatively regulated by temperatures of $<37°C$ and increased levels of nicotinic acid and $MgSO_4$, and under these conditions these regulated virulence determinants are not expressed (268). The *vir* region has been sequenced, revealing three open reading frames (B. Arico et al., manuscript in preparation). Interestingly, two of the predicted polypeptide sequences of *vir* exhibit homology (but retain several differences) to several two-component bacterial regulatory systems which transcriptionally regulate several genes. These systems are used to respond to environmental stimuli and control chemotaxis (*cheA/cheY*), phosphate response (*phoR/phoB*), and osmotic response (*envZ/ompR*) in *E. coli*, sporulation in *Bacillus subtilis* (*spoA*), and tumor formation in *Agrobacterium tumefaciens* (*virA/virG*) (137, 161a, 174, 206, 273). Although the roles of nicotinic acid and $MgSO_4$ in *vir* regulation have not been determined, these chemicals and temperature probably signal to these bacteria the nature of their environment, allowing these organisms to adapt to life in a specific milieu.

Temperature is an important regulatory cue for microbial pathogens, particularly for those infectious agents which usually experience environmental temperatures lower than the ranges found within their hosts. *Shigella* and *Yersinia* species are both examples of species which are subject to this type of regulation. Synthesis of plasmid-encoded *Shigella* virulence genes is also negatively regulated by a chromosomal factor, *virR*, at 30°C but not at 37°C (151).

Several outer membrane proteins of *Yersinia* species are tightly regulated by temperature and calcium levels and are expressed only at 37°C in low levels of Ca^{2+} (49, 244). Pollack et al. constructed a β-galactosidase fusion with one of these genes (*yopK*) in *Y. pestis* and examined its regulation in bacteria residing within human macrophages (195). These workers found that this gene was expressed in the phagolysosomal environment in macrophages, indicating that low levels of Ca^{2+} present in this locale may serve as a signal to the invading bacterium that it is entering a potentially hostile environment. Other workers have found that iron can also affect expression of high-molecular-weight outer membrane proteins in *Yersinia* species (40), indicating that these bacteria respond to various stimuli in order to adapt appropriately to life within a particular host.

Mekalanos and co-workers have characterized a gene (*toxR*) from *Vibrio cholerae* whose product is a global regulator. It transcriptionally regulates the expression of cholera toxin, several products needed for pilus synthesis,

and several other unidentified membrane proteins (165, 253). ToxR is a transmembrane protein which shares homology with the previously mentioned two-component regulatory systems and is affected by multiple physiological and nutritional signals, including pH, temperature, and osmolarity (165). Again, V. cholerae probably uses this sensory/regulatory system (and perhaps others) to discriminate its natural environment (estuaries and brackish-water habitats) from other surroundings, including the human gut. Thus, it seems that the global regulation of bacterial virulence determinants uses mechanisms adapted from the nonparasitic brethren of the pathogens. This is another example in which diverse pathogens utilize a common theme to achieve a similar goal. Each microorganism must obey the distinctive procaryotic structural constraints for the placement of sensing proteins on the bacterial surface as well as for signal transduction of received stimuli. Yet, each pathogen has uniquely designed its cascade of responsive genes to meet its particular goals for survival and multiplication in the environments that it commonly confronts during its life cycle.

We have found that Salmonella species produce several new proteins when they interact with epithelial cell surfaces (167a). Conversely, the synthesis of several other proteins is inhibited by this interaction. Salmonella mutants which no longer synthesize these induced proteins do not adhere or invade and, at least with Salmonella typhimurium, are avirulent. If epithelial surfaces are modified by neuraminidase or trypsin treatment, no new proteins are induced, and Salmonella cells do not bind to these surfaces. We think that Salmonella spp. interact weakly with epithelial surfaces, and this interaction then triggers the de novo synthesis of several bacterial proteins required for bacterial adherence and invasion and possibly for intracellular survival and replication. We suppose that this response by Salmonella spp. to the eucaryotic cell surface is singularly adapted to the requirements for life within their animal hosts, but not in the external environment.

The study of genes regulated by intracellular environments is in the early stages. As mentioned above, another regulatory locus of Salmonella typhimurium, phoP, is essential for survival within macrophages (62a; J. Mekalanos, personal communication). The product of this gene (PhoP) may use intracellular signals to control virulence genes required for life within a phagolysosome.

Chlamydia spp. may be able to sense the oxidative potential of their environment, as this potential differs between extracellular and intracellular environments. These organisms contain several cysteine-rich outer membrane proteins, including the major porin, and the cysteine residues of these proteins can be oxidized or reduced, depending on the environment (15). In elementary bodies, the infectious form of Chlamydia spp., these proteins are probably oxidized and are cross-linked by disulfide bonds, providing stability to the elementary body. After phagocytosis, these organisms encounter a "reducing" environment inside host cells, which may cause pores to open by reducing disulfide bonds, allowing nutrient exchange and growth. Should the host cell become physiologically exhausted, oxidation of the disulfide bonds occurs, preparing the bacterium for release from the cell.

Coxiella burnetii, the causative agent of Q fever, is an obligate intracellular parasite which requires low-pH environments to multiply. This requirement is met within phagolysosomes, as eucaryotic cells usually acidify endosomes before phagosome-lysosome fusion occurs (158). This organism senses a drop in pH as the endosome is acidified

and initiates intracellular multiplication. Inhibition of endosome acidification by a variety of means inhibits intracellular Coxiella multiplication, mediated in all likelihood by a pH-sensing regulator (90).

The study of gene expression in intracellular organisms is complicated by the presence of viable host cells. Specific bacterial gene fusions can be constructed and assayed. If the parasite does not require host cell protein synthesis for its intracellular existence, specific drugs can be used to inhibit host protein synthesis, and bacterial protein synthesis can be monitored by pulse-labeling bacterial proteins. Preliminary results in our laboratory indicate that such an approach can be applied to examine specific macromolecular biosynthesis by intracellular Salmonella spp. As more techniques are developed and as more intracellular pathogens are studied within their natural host environments, more examples of this type of regulated staging of virulence gene expression will probably arise.

EVOLUTION OF PATHOGENIC MECHANISMS

Pathogenic organisms are not accidents of evolution. Rather, they represent the result of microbial adaptation to a particular survival strategy which entails growth on or within another (usually more highly evolved) organism. Moreover, the evolution of pathogens and of pathogenic traits continues to be dynamic. For example, previously unrecognized human pathogens or new variants of well-recognized pathogenic microbial species have arisen to take advantage of new environments or new opportunities that have arisen by deliberate human action. Such "diseases of human progress" (60), represented by Legionnaires disease, toxic shock syndrome, a myriad of iatrogenic infections, and epidemics of sexually transmitted disease, including acquired immunodeficiency syndrome, all testify to the remarkable adaptability of pathogenic microorganisms and their capacity to exploit and quickly adapt to any breakdown in the defense systems of their hosts.

Clonality

Not all strains of a virulent bacterial species are equally pathogenic. Examination of the genetic organization of pathogens, opportunistic pathogens, and non-pathogens has been of use in determining the origins of pathogenic bacteria and also the relationships between pathogens. Several techniques exist for probing the genetic composition of microorganisms, including DNA hybridization studies, comparison of protein and nucleic acid sequences, and, more recently, multilocus enzyme electrophoresis. The last technique measures the electrophoretic mobility of several metabolic enzymes; specific electrophoretic types can be assigned to groups of strains, allowing the relationships between several strains to be examined (225, 226).

Selander and co-workers have demonstrated that most natural bacterial populations consist of several discrete clonal lineages, indicating that the rate of recombination between different strains or different species is low. By examining various pathogenic populations, including Bordetella pertussis, H. influenzae, Legionella sp., Neisseria meningitidis, Salmonella sp., and Streptococcus sp., these workers found that most diseases are caused by a small proportion of the total number of clones for these species (226; R. K. Selander and J. M. Musser, in B. H. Iglewski and V. L. Clark, ed., Molecular Basis of Bacterial Pathogenesis, in press). For example, of the 104 clonal types identified

for *H. influenzae* type b, 6 were recovered from 81% of infected patients with "invasive" disease (173; Selander and Musser, in press). Although clonality is true for most pathogenic bacterial strains, two notable exceptions were found. *Neisseria gonorrhoeae* and *P. aeruginosa* did not follow this pattern. These two organisms use chromosomal recombination and probably transformation to increase their genetic diversity of virulence factors. *Salmonella* species are usually clonal, but there is some diversity between members of the same serogroup which is best explained by horizontal genetic transmission and recombination of chromosomal genes (18).

Haemophilus aegyptius (*H. influenzae* biogroup aegyptius) is an organism which has recently been recognized as a serious human pathogen (26, 27). Over a century ago, Koch described a small bacillus associated with purulent conjunctivitis in Egypt, and this organism was named *Haemophilus aegyptius*. This organism is very similar to *H. influenzae* biotype III based on DNA hybridization studies (41). Very recently, this organism was recognized as causing Brazilian purpuric fever (BPF), a new fulminant pediatric disease characterized by fever, shock, and death (26, 27). Preceding BPF is a purulent conjunctivitis which resolves before onset of the fever. The Brazilian Purpuric Fever Study Group has studied several isolates of *H. influenzae* biogroup aegyptius to determine the epidemiology of BPF. By examining the ribosomal RNA hybridization patterns of 92 isolates of this species from various sources, these workers identified 15 ribosomal RNA gene restriction fragments, only 2 of which were associated with strains causing BPF (108). Furthermore, all 15 isolates from BPF cases were identical in their DNA relatedness and were all of a single multilocus electrophoretic type and sodium dodecyl sulfate gel electrophoresis type, and all harbored the same 24-MDa plasmid (28). In contrast, the control strains (not associated with BPF) were of several different biotypes, and none contained the same 24-MDa plasmid. Thus, a specific clonal lineage of this species is responsible for all cases of BPF.

Although the reasons for clonality of pathogenic species are not clear, it may be that only a given population has all of the necessary virulence determinants. This may explain the sudden appearance of a single clonal type of *H. influenzae* biogroup aegyptius which is now capable of causing BPF. Although this organism has been recognized for over a century as a conjunctivitis-causing organism, the sudden acquisition of one or more virulence determinants (perhaps introduced and encoded by the unique 24-MDa plasmid) presumably transformed this clonal lineage into a serious pediatric pathogen which causes BPF.

Another example of an association between a specific virulence determinant and disease is found within *Yersinia* species. It is known that only a few of the many serotypes of *Yersinia* spp. are virulent and that virulence of *Y. enterocolitica* is linked to the presence of a single invasion gene, *ail* (see above) (163). Soil and other nonpathogenic *Y. enterocolitica* isolates do not encode this gene, while the opposite is true of disease-associated isolates. Thus, only clonal populations containing this gene are isolated from patients, as this gene and its product are presumably required for infection and disease.

Mechanisms of Genetic Exchange

Although only a small number of clones within a given species cause disease, the existence of many clonal lineages for most species suggests that there are mechanisms for the exchange of genetic material between clones and even species. The ability to "sample" genetic elements from other clonal lineages under selective pressure would enhance the survivability of a bacterium (37). Several methods exist to exchange and rearrange genetic material between bacteria, including conjugation, transposition, and transduction (60). Virulence factors are often encoded on these mobile genetic elements, i.e., plasmids, transposons, and bacteriophages. The placement of virulence factors on mobile genetic elements leaves the integrity of the chromosome intact, while allowing the organism to increase its genetic diversity. These elements can often be transferred between various bacterial species, as has been witnessed for antibiotic resistance plasmids. Furthermore, antibiotic resistance genes, toxins, and other virulence factors are often flanked by transposable elements which allow the DNA encoding the virulence factor to integrate into a recipient's genome. Several virulence determinants are flanked by a transposon, so that an entire bloc of genes can be transferred via a plasmid to another strain, sometimes converting a nonpathogen into a pathogen. These blocs of virulence determinants are usually strongly conserved over time and among diverse bacterial species, perhaps indicating the selective advantage conferred by these virulence determinants.

Nonpathogenic bacteria are usually devoid of virulence determinants encoded by pathogenic organisms belonging to the same species. It is rare that sequences encoding remnant inactive or truncated forms of a virulence determinant are found in nonpathogenic organisms. This is exemplified by *ail* in *Y. enterocolitica* (163). (The other *Yersinia* invasion gene, *inv*, is found in various inactive forms in nonpathogenic species, but this gene is not completely correlated with virulence.)

CONCLUSIONS

Microbial pathogenicity transcends a number of complex disciplines. However, unifying this field are several shared tactics that pathogenic organisms must follow to sustain themselves and to overcome host barriers. The mechanisms used are diverse, but common themes continually emerge as we examine the biology of even the most diverse pathogens. Microorganisms must enter the host by a limited number of routes. Interactions with host surfaces are usually involved at, or soon after, entry. The distribution of the eucaryotic receptor to which the bacterium adheres usually dictates the site at which the organism will successfully colonize. Bacterial chemotaxis may also participate in these early stages of infection, propelling the organisms to desired locations. The mechanisms of bacterial adherence are diverse, but usually involve receptor-ligand interactions.

Colonizing organisms may cause damage to their host through toxins, although the roles that toxins play in pathogenesis are not always clear. To avoid the host immune onslaught, bacteria may alter their surfaces frequently. Alternatively, they may seek shelter inside eucaryotic cells, which in turn offer a rich new environment and a shuttle vehicle with which to disseminate throughout the host. Most organisms appear to enter eucaryotic cells by capitalizing on preexisting internalization pathways, but they may alter these pathways for their benefit.

The study of intracellular organisms has often been a frustrating field, but the recent advances in techniques and basic knowledge of eucaryotic cells should enlarge our knowledge about host-pathogen relationships. We are beginning to address the expression of bacterial genes which are

activated as these organisms interact with eucaryotic cells. This investigation has led to promising data which suggest that organisms adapt to life within a host by altered expression of several genes. Identification and characterization of these genes will provide new insights into microbial pathogenesis.

ACKNOWLEDGMENTS

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